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Feeding and respiration by giant barrel sponges across a gradient of food abundance in the Red Sea

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Abstract

While sponges are well-known to be suspension feeders, consumption of dissolved organic carbon (DOC) has recently been highlighted as a mechanism whereby sponges may avoid food limitation. Further, the sponge-loop hypothesis proposes that sponges consume DOC and then release shed cellular detritus back to the reef benthos. We examined the carbon flux mediated by the giant barrel sponge, *Xestospongia testudinaria*, on reefs in the Red Sea across an inshore–offshore gradient that had previously been proposed to affect sponge nutrition in other parts of the tropics. Seawater samples were collected from the incurrent and excurrent flow of 35 sponges. Concentrations of total organic carbon and its components, DOC, live particulate organic carbon (LPOC), and detritus, were all significantly higher in incurrent seawater on inshore than offshore reefs. The diet of *X. testudinaria* was comprised primarily of DOC and detritus, with mean values across all reef sites of 61.5% DOC, 34.6% detritus, and 3.9% LPOC. Across the inshore–offshore gradient, there was evidence (1) of a threshold concentration of DOC ($\approx$ 79 μmol C $L_{seawater}^{-1}$) below which sponges ceased to be net consumers of DOC, and (2) that sponges on offshore reefs were food limited, with a mean carbon deficit relative to sponges on inshore reef sites. Sponges on offshore reef sites exhibited higher pumping rates, perhaps indicating optimal foraging for POC. As previously demonstrated for *Xestospongia muta,* and contrary to the sponge-loop hypothesis, there was no evidence that *X. testudinaria* returned DOC to the benthos in the form of detritus.

In recent years, there has been increasing interest in the ecological roles of sponges on coral reefs. Among the reasons for this include: (1) reports of increasing sponge abundance on some reefs (Bell et al. 2013; Loh and Pawlik 2014; de Bakker et al. 2017), (2) greater recognition of the ability of sponges to disrupt boundary flow on the reef and mix the water column (Reiswig 1971; Weisz et al. 2008; McMurray et al. 2014), (3) verification that many sponge species have the capacity to feed on dissolved organic carbon (DOC) as part, or most, of their diet (Yahel et al. 2003; de Goeij et al. 2013; McMurray et al. 2018), and (4) the discovery that sponges are an important source of nitrogen-based nutrients to the reef ecosystem (Diaz and Ward 1997; Southwell et al. 2008; Morganti et al. 2017). Individually, these research topics are interesting and have developed rapidly, but collectively, they have sparked new ideas about the ecosystem function of sponges on coral reefs, including the sponge-loop hypothesis for the retention of carbon by sponges through the uptake of DOC and production of cellular detritus (de Goeij et al. 2013; Rix et al. 2016) and the vicious circle hypothesis for the relative lack of resilience of Caribbean coral reefs (Pawlik et al. 2016).

Parallel to the foregoing developments, a debate has emerged about whether Caribbean coral reef sponges are food limited and how this may influence their distributions and abundances (Pawlik et al. 2018). Based on correlations between the concentration of picoplanktonic food and sponge abundance, tube elongation, and size, Lesser (2006) and Trussell et al. (2006) concluded that sponge populations are controlled principally by bottom-up processes. This assertion was challenged by Pawlik et al. (2013) who conducted a series of manipulative sponge growth experiments that excluded sponge predators as part of the experimental design and found evidence that sponge predation, but not food abundance, affected sponge growth. Subsequent deliberation has focused on the importance of planktonic food availability (Lesser and Slattery 2013; Slattery and Lesser 2015) or predation (Pawlik et al. 2015*a*,*b*) in structuring sponge

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Additional Supporting Information may be found in the online version of this article.

communities on Caribbean reefs. Surprisingly, however, the aforementioned support for food limitation of sponges only considered food available in the form of picoplankton and did not consider the potential contribution of DOC and detritus to the sponge diet (Pawlik et al. 2018).

The importance of DOC as a source of nutrition for some sponges has been suspected since the work by Reiswig (1974, 1981) revealed that particulate organic carbon (POC) could only account for 14–25% of the respiratory requirements for the sponges *Aplysina fistularis* and *Verongia reiswigi*. More recently, DOC uptake has been confirmed for an increasing number of species (e.g., Yahel et al. 2003; Hoer et al. 2018; McMurray et al. 2018), including both encrusting and emergent (large and nonencrusting) forms, and those having high and low abundances of microbial symbionts (HMA and LMA sponges, respectively) (Pawlik et al. 2018). This is significant, as >90% of the total organic carbon (TOC) available in seawater is typically in the form of DOC, and DOC frequently constitutes >70% of TOC uptake by sponges (*see* Pawlik et al. 2018, for review).

For some sponge species, the magnitude of DOC uptake is such that TOC consumed greatly exceeds respiratory demands; for example, the encrusting sponge *Halisarca caerulea* was found to respire only 39–45% of TOC consumed (de Goeij et al. 2008), with the balance of carbon found to be allocated to rapid cell turnover and shedding (de Goeij et al. 2009). Importantly, further work on DOC uptake and carbon flux by *H. caerulea* and three other cryptic, encrusting sponge species suggested that sponge-mediated recycling of DOC released by primary producers (e.g., reef-building corals and macroalgae) as particulate detritus that is then available to higher trophic levels may sustain the high productivity of coral reef ecosystems; a process termed the "sponge-loop" (de Goeij et al. 2013). A link between DOC uptake and detritus production has been established for additional cryptic species (Alexander et al. 2014; Rix et al. 2016; Rix et al. 2017). However, the generality of the sponge-loop across the Porifera remains unclear, as the emergent species studied to date have lacked as large a discrepancy between rates of DOC uptake and respiration (Yahel et al. 2003; Hoer et al. 2018), and, with the exception of the branching sponge *Negombata magnifica* (Rix et al. 2018), emergent sponge species have not been found to produce significant quantities of detritus (McMurray et al. 2018).

Despite the importance of DOC to the diets of some sponge species, wide intraspecific variability in DOC uptake by sponges has been reported (Archer et al. 2017; Hoer et al. 2018; McMurray et al. 2018). The composition and nutritional value of the DOC pool is highly heterogeneous (Hansell and Carlson 2002), and sponges are generally thought to feed on the labile, rather than refractory, fraction of DOC (Yahel et al. 2003; de Goeij et al. 2008). While primary producers are a large source of labile DOC on coral reefs (Wild et al. 2004; Haas et al. 2010), sponges may consume macroalgal-derived DOC at higher rates than coral-derived DOC (Rix et al. 2017). Additionally, there is increasing evidence that sponge-mediated DOC flux may vary as a direct function of the concentration of DOC in ambient seawater and that there may be a threshold concentration of DOC uptake below which sponges cease to be net consumers of DOC (Mueller et al. 2014; Archer et al. 2017; McMurray et al. 2017; Morganti et al. 2017). Thus, although DOC constitutes the largest pool of TOC potentially available to sponges, there are conditions under which food limitation of sponges may be expected to occur, including on reefs where DOC quality or concentration is insufficient (Pawlik et al. 2018).

Studies of the abundance and biomass of sponges across a gradient from inshore to offshore reefs in the Pacific and Atlantic Oceans were among the first to suggest that food availability may influence the abundance and nutritional symbioses of sponges on coral reefs (Wilkinson 1987; Wilkinson and Cheshire 1990). Specifically, using a series of transect surveys, Wilkinson (1987) and Wilkinson and Cheshire (1990) found that sponge biomass was five to six times greater on Caribbean reefs relative to those on the Great Barrier Reef (GBR), Australia, and that sponge biomass was highest on reefs adjacent to land and decreased across the shelf to oceanic reefs. Furthermore, there were no foliose phototrophic sponge species (with a plant-like growth form and reliant on photosynthetic microsymbionts for most of their nutrition) in the Caribbean and a pattern of increasing abundance of phototrophic species across the inshore–offshore gradient on the GBR, suggesting that food limitation was driving the evolution of the nutritional symbiosis (Wilkinson and Cheshire 1990). Therefore, while patterns of abundance and nutritional symbiosis were attributed to inter-ocean and cross-shelf gradients of food availability (Wilkinson 1987; Wilkinson and Cheshire 1990), the relative availability of food for sponges across these gradients was not explored.

In this study, we measured carbon flux and sponge feeding of *X. testudinaria* across an inshore–offshore gradient in the Red Sea using methods similar to those recently employed for *Xestospongia muta* in the Caribbean (McMurray et al. 2016; McMurray et al. 2018) to test the implicit hypothesis raised by Wilkinson and coworkers that sponges may be affected by a cross-shelf gradient of food availability (Wilkinson 1987; Wilkinson and Cheshire 1990). Additionally, we obtained measurements of dissolved oxygen (DO) in the incurrent and excurrent seawater for each sponge to provide a comparison of oxygen availability and sponge respiration rates across reef sites. Finally, we wanted to test the components of the sponge-loop for an emergent sponge on Red Sea coral reefs.

Methods

Carbon flux was quantified for *Xestospongia testudinaria* in the Saudi Arabian Red Sea on reefs near Thuwal (3 reefs) and south of Al-Lith in the Farasan Banks (14 reefs; Supporting Information Fig. S1) in May 2017. Reefs were classified by distance from shore: inshore reefs were less than 15 km from shore (7 reefs) and offshore reefs were greater than 15 km from shore (10 reefs). The 15-km dividing distance separated

reefs that were on the land side of elongate shallow patches of sand and reef from those that were outside of these patches. A total of 35 sponges were sampled at depths of 10–21 m, with 16 sponges sampled on inshore reefs and 19 sponges sampled on offshore reefs.

Suspension feeding by *X. testudinaria* was investigated following the methods of McMurray et al. (2016). For each sponge, incurrent (ambient) seawater was collected by slowly filling a 2-liter Nalgene bottle with the mouth 3–6 cm from the middle of the external sponge surface, whereas excurrent seawater was sampled simultaneously by drawing water with multiple 100 mL syringes, placing each syringe tip 3–6 cm from the internal sponge wall in the inner empty space of the sponge (atrium), for a total sample of 1.5–1.6 liters of seawater. Seawater samples were collected at a rate slower than that of excurrent flow to minimize any potential contamination of samples with ambient seawater that had not passed through the sponge ($\approx$ 0.1–0.2 L min^{-1}); therefore, samples represented approximately 10–20 min of sponge feeding. To quantify total POC, each sample was filtered via vacuum at low pressure with a handpump through a 100 μm mesh and a precombusted (500°C for 4 h) 0.7-μm GF/F glass fiber filter; for incurrent samples, 1 liter of seawater was processed, whereas the entire pooled 1.5–1.6 liters of each excurrent seawater sample was filtered. Filters were individually wrapped in aluminum foil and frozen until analysis. To quantify DOC, 20 mL of the filtrate from each sample was transferred to an US Environmental Protection Agency (EPA) precleaned glass vial (Traceclean, VWR International), acidified in the field with 100 μL of 50% phosphoric acid, and stored at 4°C until analysis. DOC concentrations were measured using high-temperature catalytic oxidation with a Shimadzu TOC-L TN analyzer. Calibration was achieved with standards diluted from a stock solution of potassium hydrogen phthalate and both standards and deep seawater consensus reference material (batch 9, lot #09-09, Hansell Laboratory, University of Miami, RSMAS) were interspersed with samples for quality assurance and control. Each seawater sample was run in duplicate and each analysis tube was injected three to five times for a coefficient of variance <1.5%. The approximate analytical precision of the instrument was 2 μmol C $L_{seawater}^{-1}$. POC was measured using a Flash 2000 CHNS/O Elemental analyzer after filters were dried at 50°C and subsequently exposed to hydrochloric acid fumes for 24 h. All glassware and aluminum foil used to process samples was combusted prior to use and all plastic used for sample collection was acid washed before use (Tupas et al. 1994).

To quantify live POC (LPOC), 5 mL samples of incurrent and excurrent seawater were collected as described above using 5 mL syringes. Samples were fixed with 1% paraformaldehyde + 0.05 mL glutaraldehyde (final concentration) in cryovials and, after 10 min in the dark, quickly frozen in liquid nitrogen and stored at –80°C until analysis. Phytoplankton (*Prochlorococcus* [Pro], *Synechococcus* [Syn], and photosynthetic picoeukaryote and nanoeukaryote [Euk]) in seawater samples were enumerated using a BD FACSCanto II Flow Cytometer using a syringe pump. Population geometric mean properties (scatter and fluorescence) were normalized to 1.0 μm fluorescent latex beads. Picophytoplankton were classified based on their characteristic flow cytometric signatures (Cavender-Bares et al. 1998; Lindström et al. 2002). Bacterioplankton (high-nucleic acid bacteria [HNA] and low-nucleic acid bacteria [LNA]) were similarly quantified by staining samples with Sybr Green-I as previously described (Marie et al. 1997), and relative cellular DNA quantified assuming stoichiometric dye binding. Each sample was run until either 10,000 events (cells counted) were reached or 5 min had elapsed. Flow cytometer flow rates were quantified by measuring the changes in mass of 1 mL water samples after 5 min runs. Biovolume was determined using semiempirically determined conversion factors based on changes in side scatter (SSC) signals (*see* details in Calvo-Díaz and Morán [2006]), which were converted into carbon content using known values found in the literature for picophytoplankton (Worden et al. 2004) and heterotrophic bacteria (Gundersen et al. 2002). Due to difficulties in quantifying detritus via flow cytometry, the detrital carbon in each seawater sample was estimated as the difference of total POC and LPOC. A caveat to this approach is that detritus estimates may include nondetrital material <100 μm that were not within the range of detection of the flow cytometer.

Following seawater collection, the velocity of excurrent seawater at the centerline of each sponge was determined by using underwater camera video of the movement of dye fronts in excurrent flow (Savarese et al. 1997; Weisz et al. 2008). A ruler was held parallel to the central axis of each sponge and a video camera was used to record the vertical movement of small volumes of fluorescein dye that were injected into the osculum of the sponge using a syringe. Videos were later analyzed frame-by-frame using Tracker (version 4.97; Open Source Physics) video analysis software to quantify the vertical velocity of excurrent seawater at the centerline. Studies of the congener *X. muta* suggest that barrel sponge excurrent velocity profiles are typically nonuniform (parabolic) (McMurray et al. 2014); therefore, the mean excurrent velocity across the planar area of the osculum of each sponge was estimated as 0.5 times the excurrent velocity measured at the osculum centerline. The pumping rate for each sponge was then calculated as the product of the mean excurrent velocity and the area of the sponge osculum.

Seawater DO concentrations were measured with a PME MiniDOT logger, which recorded DO concentrations and seawater temperatures every minute. For each sponge, incurrent DO was measured over an approximately 3 min interval with the logger sensor positioned adjacent to the external sponge surface; subsequently, excurrent DO was measured by positioning the sensor inside of the osculum of each sponge for approximately 4 min. While incurrent and excurrent DO concentrations were not measured simultaneously (3 min apart), incurrent DO varied very little over the sampling interval (average range = 0.03 mg L^{-1}) that preceded excurrent DO

measurements; thus, incurrent DO measurements provide a good approximation of the DO available to sponges during excurrent sampling. Oxygen measurements were not obtained for two offshore sponges due to operator error; therefore, oxygen measurements were collected for a total of 16 sponges on inshore reefs and 17 sponges on offshore reefs. After sampling, the dimensions of each sponge were measured with a flexible plastic measuring tape, and sponge biomass estimates were obtained by approximating the morphology of *X. testudinaria* as a frustum of a cone (McMurray et al. 2008).

Sponge specific filtration rates, or carbon flux (μmol C s^{-1} L_{sponge}^{-1}), of DOC, LPOC, and detritus were calculated as:

$$C_{flux} = \frac{(C_{in} - C_{ex}) \times Q}{V_{sponge}}$$

where C_{in} and C_{ex} are the incurrent and excurrent concentrations of each carbon pool (μmol C $L_{seawater}^{-1}$), Q is the sponge pumping rate (L s^{-1}), and V_{sponge} is sponge tissue volume (L). For all statistical comparisons, Levene's test was used to test for homogeneity of variances and the goodness-of-fit test was used to test for normality. Ordinary least square regression was used to examine the relationship between $\log_e$-transformed incurrent food concentration and distance from shore, which was determined using Google Earth with the global positioning system coordinates of each site, for each food type. Paired *t*-tests were used to compare the concentrations of carbon in incurrent and excurrent seawater to test whether sponges were net consumers (or producers) of each food type. A one-way analysis of covariance (ANCOVA) was used to test for differences in $\log_{10}$-transformed pumping rates between inshore and offshore reefs, with sponge volume as the covariate and reef type as a fixed factor. Because homogeneity of variances or normality was not confirmed, Scheirer–Ray–Hare tests (two-way nonparametric analysis of variance; Sokal and Rohlf 1995) were used to test for differences in (1) the concentrations of ambient carbon between carbon pools (i.e., DOC, LPOC, and detritus) and reef types (inshore vs. offshore); (2) ambient picoplankton cell abundances between LPOC fractions (i.e., Pro, Syn, Euk, HNA, and LNA) and reef types; (3) percentages of total LPOC in the form of picoplankton between LPOC fractions and reef types; and (4) specific filtration rates between carbon pools and reef types. Post hoc multiple comparisons were completed with the Steel–Dwass test. Ordinary least squares regression was used to assess the relationship between specific filtration rates and $\log_e$-transformed incurrent carbon concentrations for each food type. Statistical analyses were performed using JMP Pro 13 (SAS Institute) and SPSS Statistics (version 22 for Windows; IBM) statistical software. All means are reported with ± standard error unless otherwise stated.

Results

A total of 35 *X. testudinaria* were sampled, with 16 sponges sampled on inshore reef sites and 19 sponges sampled on offshore reef sites. Sponge volume did not significantly differ between reef site types (Wilcoxon two-sample test; $U_s = 229$, $p = 0.053$), and mean sponge volumes were 3.37 ± 0.61 and 5.77 ± 0.97 on inshore and offshore reefs, respectively. ANCOVA analysis of $\log_{10}$-transformed pumping data indicated that sponges on offshore reefs pumped seawater significantly faster (0.104 ± 0.016 L s^{-1} L_{sponge}^{-1}) than sponges on inshore reefs (0.063 ± 0.006 L s^{-1} L_{sponge}^{-1}; $F_{1,32} = 7.13$, $p = 0.012$; Table 1; Supporting Information Fig. S2). Pumping efficiencies of *X. testudinaria* were similar on both reef types ($t = 0.03$, df = 31, $p = 0.97$), with sponges transporting 4.40 ± 0.73 and 4.43 ± 0.38 liters of seawater per mL DO consumed on inshore and offshore reef sites, respectively.

Ambient (incurrent) TOC available to sponges ranged from 59.1 to 222.1 μmol C $L_{seawater}^{-1}$ and was significantly higher on inshore than offshore reef sites ($t = 5.31$, df = 33, $p < 0.0001$; Fig. 1). When TOC was divided into the three food types (DOC, LPOC, and detritus), there was a significant difference in the concentration of carbon available as each food type ($H = 45.92$, df = 2, $p < 0.0001$), and there was significantly more carbon available on inshore than on offshore reefs ($H = 4.67$, df = 1, $p = 0.031$; Fig. 1). Pairwise comparisons revealed that there was more carbon available in the form of DOC relative to detritus and LPOC ($p < 0.0001$ for both tests) and more detritus relative to LPOC ($p < 0.0001$; Fig. 1; Table 1). When the food concentration data were analyzed as a function of distance from shore as a continuous variable, there was a significant inverse relationship between ambient food concentration and distance from shore for each food resource (TOC, $r^2 = 0.58$, $p < 0.0001$; DOC, $r^2 = 0.55$, $p < 0.0001$; LPOC, $r^2 = 0.33$, $p = 0.0003$; detritus, $r^2 = 0.27$, $p = 0.0015$; Supporting Information Fig. S3).

Cell abundance differed between picoplankton types ($H = 35.63$, df = 4, $p < 0.0001$), but there was no difference in cell abundance between inshore and offshore reef sites ($H = 1.26$, df = 1, $p = 0.26$; Supporting Information Table S1), and mean total ambient (incurrent) picoplankton cells (i.e., all types combined) did not significantly vary between reef site types ($t = 1.94$, df = 33, $p = 0.062$). The percentage contribution of each food type to total ambient LPOC significantly differed between picoplankton types ($H = 25.43$, df = 4, $p < 0.0001$) and between inshore and offshore reef sites ($H = 8.05$, df = 1, $p = 0.005$; Supporting Information Table S1). Pairwise comparisons between the LPOC food types indicated that the percentage of LPOC in the form of Syn was significantly higher than that of Pro, Euk, LNA, and HNA ($p < 0.0001$ for all comparisons) and that the percentage contribution of Pro was significantly lower than Euk ($p = 0.0002$), Syn ($p < 0.0001$), LNA ($p = 0.0009$), and HNA ($p = 0.0006$; Supporting Information Table S1).

Sponges on both inshore and offshore reef sites consumed significant quantities of LPOC and detritus, whereas consumption of DOC was only significant for sponges on inshore reefs (Table 1). Of the 35 sponges that were investigated, 23 were net consumers of DOC, with 75.0% and 57.9% of the individuals found to consume DOC on inshore and offshore reefs,

Table 1. Mean ± standard error sponge volume, sponge pumping rates, incurrent carbon, and carbon fluxes for the sponge *X. testudinaria* on inshore and offshore reefs in the Red Sea. Food types were DOC, LPOC, and detritus (DET). Percentage of total carbon uptake is the percentage contribution of each food type to the specific filtration rate for TOC.

Reef type	Sponge volume (liter)	Pumping rate (L s^{-1} L_{sponge}^{-1})	Food type	*n*	Incurrent carbon (μmol C $L_{seawater}^{-1}$)	Carbon consumed (μmol C $L_{seawater}^{-1}$)	Specific filtration rate (μmol C s^{-1} L_{sponge}^{-1})	% total carbon uptake
Inshore	3.37 ± 0.61	0.063 ± 0.006	DOC	16	137.36 ± 10.47	17.53 ± 6.74*	1.016 ± 0.370	69.1
			LPOC	16	0.73 ± 0.06	0.62 ± 0.06*	0.038 ± 0.005	2.6
			DET	16	10.79 ± 0.94	7.18 ± 0.92*	0.417 ± 0.054	28.3
Offshore	5.77 ± 0.97	0.104 ± 0.016	DOC	19	86.48 ± 4.24	2.18 ± 3.33	0.307 ± 0.365	47
			LPOC	19	0.41 ± 0.05	0.34 ± 0.05*	0.042 ± 0.013	6.5
			DET	19	6.21 ± 0.30	3.05 ± 0.34*	0.304 ± 0.047	46.5

n, number of samples.
Significant differences between incurrent and excurrent concentrations of food types are indicated by an asterisk: * $p < 0.05$, paired *t*-test.

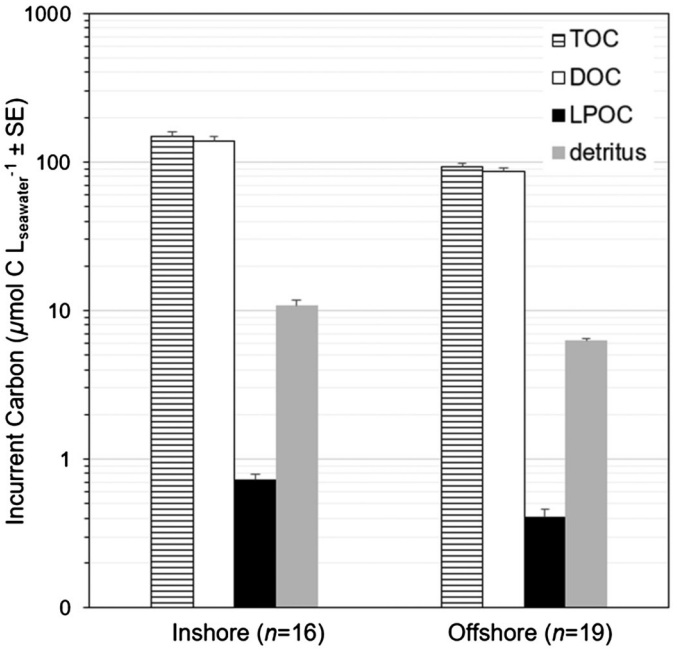

Fig. 1. Mean ± standard error concentrations of TOC, DOC, LPOC, and detritus in incurrent seawater (next to replicates of the sponge *X. testudinaria*) on inshore and offshore reefs in the Red Sea. Number of sponges sampled (*n*) indicated in parentheses for inshore and offshore reefs.

respectively. In contrast, all sponges were net consumers of LPOC, and 34 of 35 sponges were net consumers of detritus. The one individual sponge that had a negative detrital flux was from an offshore site and produced only 0.0005 μmol detrital C s^{-1} L_{sponge}^{-1}. The mean percentage of DOC, LPOC, and detritus in the diet of *X. testudinaria* for inshore reefs was 69.1%, 2.6%, and 28.3%, respectively, and 47.0%, 6.5%, 46.5% for offshore reefs (Table 1).

Specific filtration rates were significantly different between food types ($H = 13.14$, df = 2, $p = 0.001$) and tended to decrease with increasing distance from shore but were not significantly different between inshore and offshore reefs ($H = 2.0$, df = 1, $p = 0.16$; Fig. 2). Pairwise comparisons for food types indicated that the mean specific filtration rate for LPOC was significantly lower than that of detritus ($p < 0.0001$) but not DOC ($p = 0.15$), whereas sponges consumed detritus and DOC at similar rates ($p = 0.95$). There was a significant direct logarithmic relationship between specific filtration rates and incurrent food concentrations for TOC as well as for the three different food types, and incurrent food concentrations explained between 23% and 27% of the variance in specific filtration rates (TOC, $r^2 = 0.25$, $p = 0.002$; DOC, $r^2 = 0.27$, $p = 0.001$; LPOC, $r^2 = 0.25$, $p = 0.002$; detritus, $r^2 = 0.23$, $p = 0.003$; Fig. 3).

Ambient DO concentrations were significantly lower on inshore reefs relative to offshore reefs (mean = 176.6 ± 4.2 and 186.9 ± 2.2 μmol $L_{seawater}^{-1}$, respectively; $t = 2.06$, df = 15, $p = 0.034$). All sponges were found to consume DO, and excurrent DO was significantly reduced relative to ambient (incurrent)

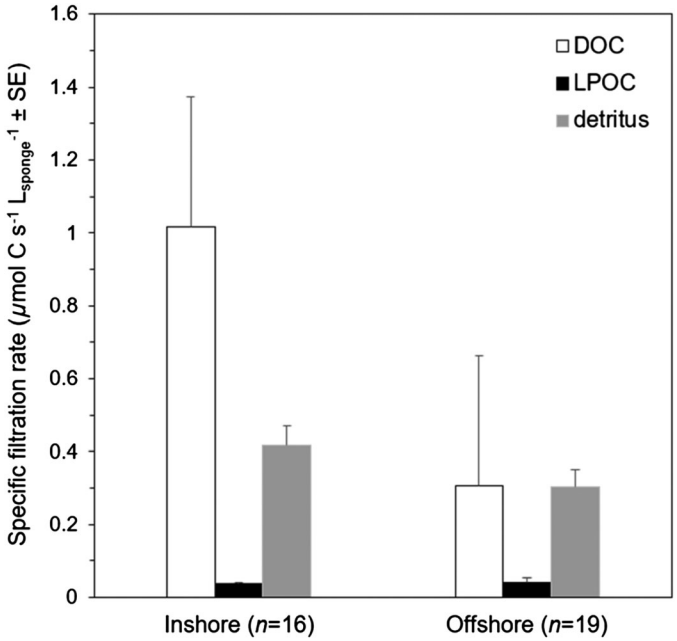

Fig. 2. Mean ± standard error specific filtration rates of DOC, LPOC, and detritus by the sponge *X. testudinaria* on inshore and offshore reefs in the Red Sea. Number of sponges sampled (*n*) is indicated in parentheses for inshore and offshore reefs.

DO (t = 14.99, df = 33, $p < 0.0001$), reflecting the respiration of the sponge and its microbiome, minus any oxygen produced by photosynthetic microbial symbionts in daylight. The retention of DO did not differ between reef site types (t = 1.58, df = 31, p = 0.12), and sponges on inshore and offshore reef sites retained 5.96% ± 0.43% and 7.28% ± 0.73% of incurrent DO, respectively. The flux of DO was similar between inshore and offshore reef sites (t = 1.39, df = 31, p = 0.17; Table 2).

There were no significant differences between the fluxes of TOC and DO on inshore or offshore reef sites (paired *t*-tests, p = 0.069 and 0.20, respectively; Fig. 4). However, the quantity of carbon needed for respiration (i.e., the respiratory quotient [RQ]) is dependent on both animal physiology and the chemical composition of the food used for oxidation, and theoretically varies from 0.7 to 1.0 mol C mol^{-1} DO (Koopmans et al. 2010). Therefore, following Hoer et al. (2018), we quantified the respiration balance for *X. testudinaria* in two ways: first by assuming a RQ of 1.0, and second by assuming a RQ of 0.7. With a RQ = 1, sponges on inshore reefs exhibited mean TOC removal in excess of respiratory DO demand of 0.65 ± 0.33 μmol C s^{-1} L_{sponge}^{-1}, with respiration accounting for 56% of the carbon acquired. On offshore reefs, TOC uptake was insufficient to meet respiratory DO demand and sponges had a mean carbon deficit of 0.48 ± 0.36 μmol C s^{-1} L_{sponge}^{-1} (Fig. 4). The ratio of mean TOC to DO demands was 1.8 and 0.59 for sponges on inshore and offshore reefs, respectively. With an RQ = 0.7, the excess carbon retained by sponges on inshore reefs increased to 0.90 ± 0.34 μmol C s^{-1} L_{sponge}^{-1}, while sponges on offshore reefs had a mean carbon deficit of 0.13 ± 0.36 μmol C s^{-1} L_{sponge}^{-1}. Considering both reef types combined, there was a significant direct relationship between DO demand and the specific filtration rate of TOC (r^2 = 0.13, p = 0.041; Supporting Information Fig. S4), reflecting greater carbon intake to meet increased respiratory demand.

Discussion

The diet of *X. testudinaria* on Red Sea reefs consisted of mostly DOC followed by detritus and then LPOC, with DOC, detritus, and LPOC comprising 61.5%, 34.6%, and 3.9%, of the sponge diet across all reef sites, respectively. The proportions of the three food types in the diet of *X. testudinaria* were similar to those reported in the diet of *X. muta* in the Caribbean: ~ 70% DOC, ~ 20% detritus, and ~ 10% LPOC (McMurray et al. 2016). The high percentage of DOC in the diet is consistent with previous studies of HMA sponges (Yahel et al. 2003; de Goeij et al. 2008; Mueller et al. 2014; McMurray et al. 2016; Hoer et al. 2018).

Although the diet of *X. testudinaria* consisted mostly of DOC, 12 of the 35 sponges were found to be net producers of DOC and 8 of these 12 were from offshore reef sites. The type and available concentration of DOC are believed to affect its uptake rates (McMurray et al. 2016; Rix et al. 2017). It is thought that sponges primarily consume the labile fraction of DOC rather than refractory forms (Yahel et al. 2003; de Goeij et al. 2008). Higher rates of uptake of algal- vs. coral-derived DOC by sponges were reported from the results of incubation experiments (Rix et al. 2017). Consistent with prior work, this study provides evidence that there was a threshold concentration at which DOC consumption was equivalent to DOC production by the sponge holobiont (i.e., zero net consumption) and that uptake varied directly with ambient DOC availability (Fig. 3; e.g., Mueller et al. 2014; McMurray et al. 2016, 2017; Morganti et al. 2017). As the incurrent concentration of DOC decreased from inshore to offshore, so did the consumption of DOC, consistent with the hypothesis that there is a threshold concentration (approximately 79 μmol C $L_{seawater}^{-1}$) below which sponges no longer exhibit net DOC uptake (Table 1; Fig. 3). For the 12 sponges that had negative carbon flux, 8 of which were at offshore reef sites, the average ambient DOC concentration (±SE) was 88.9 ± 8.9 μmol C $L_{seawater}^{-1}$. The ambient carbon concentration for sponges that had a positive flux for DOC was 120.6 ± 8.5 μmol C $L_{seawater}^{-1}$. Although the amount and type of DOC that can be consumed by sponges is unknown, it has been suggested that the lower limit of uptake may reflect the remaining concentration of refractory DOC after the labile DOC has been consumed, a concentration that was previously approximated at 80 μmol C $L_{seawater}^{-1}$ for Mediterranean sponge species (Morganti et al. 2017). We are unaware of any work that has characterized sponge-produced DOC, but hypothesize that sources may include sponge metabolic wastes, primary production by microbial photosymbionts, lysis of microbial symbionts

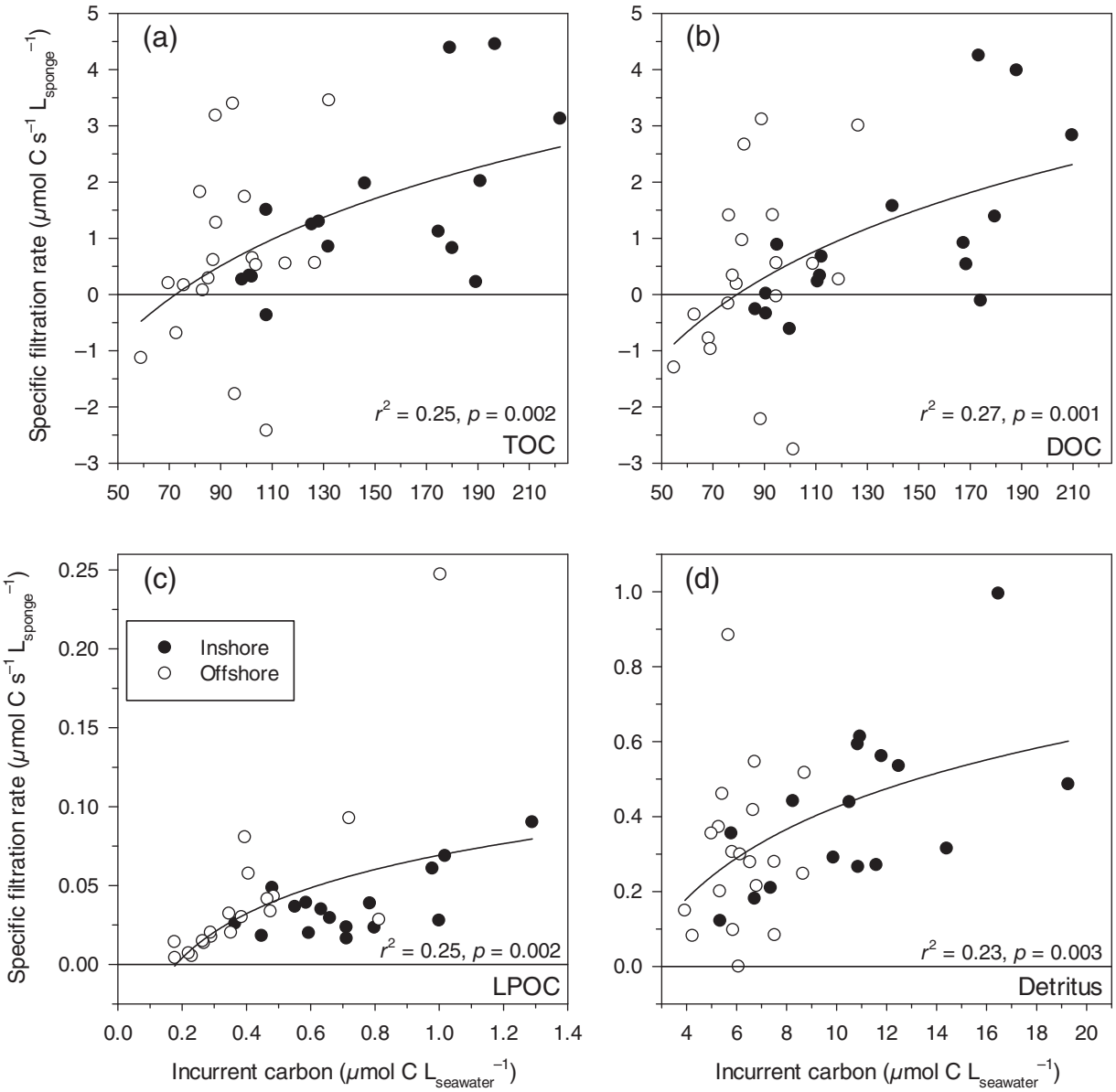

Fig. 3. Relationship between specific filtration rates of food components by the sponge *X. testudinaria* and the concentration of carbon in incurrent seawater for (**a**) TOC, (**b**) DOC, (**c**) LPOC, and (**d**) detritus for reefs in the Red Sea. Sponges at inshore (n = 16) and offshore (n = 19) reefs are marked as black dots and open circles, respectively.

by viruses, or the activities of endosymbiotic macrofauna (e.g., brittlestars, polychaetes, and shrimps).

This study supports the hypothesis that sponges on offshore oceanic reefs may be food limited, a proposal originally advanced by Wilkinson and Cheshire (1990) from studies of sponge distributions and abundances across the continental shelf of the GBR and out to the Coral Sea. The inshore to offshore gradients documented herein for the Red Sea consist of an increase in DO, a decrease in DOC and TOC, and a shift in the picoplankton that comprise the LPOC. These changes correspond to an increase in pumping rates with no change in pumping efficiency (i.e., liters of seawater pumped per milliliter of DO consumed), reduction in the specific filtration rates of sponges across the inshore to offshore gradient (Fig. 2), a decrease in the number of sponges consuming DOC (Fig. 3), an increase in DO demands, and a decrease in carbon uptake relative to DO demand (Fig. 4), resulting in mean excess carbon retention for sponges at inshore reef sites to a mean carbon deficit for sponges at offshore reef sites. Regressions of the relationship between specific filtration rates and incurrent carbon concentrations revealed that sponges had higher filtration rates for the different food types when there was more food available (Fig. 3), a relationship previously demonstrated for other sponge species (Mueller et al. 2014; Archer et al. 2017; Morganti

Table 2. Comparison of the mean ± 1 SD incurrent (ambient) availability and sponge-mediated flux of C and DO for the giant barrel sponges *X. testudinaria* and *X. muta*. Incurrent C and DO concentrations are reported as µmol C $L_{seawater}^{-1}$ and µmol O_2 $L_{seawater}^{-1}$, respectively. Flux measurements are reported as µmol C s^{-1} L_{sponge}^{-1} for DOC and POC and µmol O_2 s^{-1} L_{sponge}^{-1} for DO.

Species	Location	*n*	Incurrent DOC	DOC flux	Incurrent POC	POC flux	Incurrent DO	DO flux	Source
X. Testudinaria	Inshore reefs, Red Sea	16	137.4 ± 41.9	1.02 ± 1.48	11.5 ± 3.9	0.45 ± 0.23	177 ± 12	0.82 ± 0.51	This study
X. Testudinaria	Offshore reefs, Red Sea	19*	86.5 ± 18.5	0.31 ± 1.59	6.6 ± 1.3	0.35 ± 0.25	187 ± 7	1.16 ± 0.86	This study
X. muta	Florida, Caribbean Sea	2	89 ± 5	0.53 ± 0.42	2.4 ± 0.6	0.017 ± 0.003	177 ± 9	0.44 ± 0.11	Hoer et al. (2018)
X. Muta	Florida, Caribbean Sea	5	95.9 ± 26.9	1.8 ± 1.4	17.4 ± 2	0.87 ± 0.19	—	—	McMurray et al. (2016)
X. Muta	Florida, Caribbean Sea	32	74.2 ± 14.3	0.65 ± 0.91	11.3 ± 3.6	0.51 ± 0.25	—	—	McMurray et al. (2017)
X. Muta	Florida, Caribbean Sea	5	103.4 ± 43.6	0.19 ± 0.83	7.3 ± 3.3	0.11 ± 0.11	—	—	McMurray et al. (2018)
X. Muta	Belize, Caribbean Sea	5	102.7 ± 26.6	–0.09 ± 0.2	4 ± 1.3	0.01 ± 0.01	—	—	McMurray et al. (2018)

*n = 17 for incurrent DO and DO flux.

et al. 2017), including *X. muta* in the Caribbean (McMurray et al. 2016, 2017).

Our findings of greater oxygen demand with higher pumping rates by offshore sponges are consistent with the work by Ludeman et al. (2017), which suggests that there is an energetic cost to pumping greater volumes of seawater. This active control of pumping rates, however, was suggested to be an adaptation that might allow sponges to limit energy demands by reducing pumping during periods of low food availability (Ludeman et al. 2017). In contrast to this prediction, the present study found that sponges on offshore reefs had higher pumping rates relative to their inshore counterparts while under conditions of low food abundance. We hypothesize that higher pumping rates may partially compensate for decreased carbon uptake on offshore reefs where food availability is reduced by increasing rates of carbon flux; however, further work is required to investigate the energetic cost of pumping by *X. testudinaria* and the relationship between sponge pumping rates and foraging efficiency (McMurray et al. 2016). For example, if the quality of DOC available is low, sponges may increase their rate of pumping to capture more POC to compensate and maximize their nutritional gains. While pumping rates are also known to vary with seawater temperatures (Riisgård et al. 1993), mean in situ temperatures were similar between inshore (30.4°C) and offshore (29.8°C) sponges sampled in this study.

All barrel sponge species host photosynthetic cyanobacteria, but the nutritional role of these symbionts is thought to be minimal (López-Legentil et al. 2008; McMurray et al. 2011). For this study, sampled sponges were all similarly pigmented and under similar light levels so that the relative

Fig. 4. Mean ± standard error specific filtration rates for TOC and DO by the sponge *X. testudinaria* on inshore and offshore reefs in the Red Sea. Number of sponges sampled (*n*) is indicated in parentheses for inshore and offshore reefs.

production of photosynthate or oxygen should have been similar across all samples. Nevertheless, the role of photosymbionts in barrel sponges remains poorly understood, as they may contribute to oxygen or food production or photoprotection for the sponge.

Although the high variance between individual sponges in specific filtration rates of TOC and DO between inshore and offshore reef sites precluded a significant difference in the mean values of these metrics (Fig. 4), the analysis of respiration balance for sponges across the onshore–offshore gradient provides strong evidence that sponges on offshore reef sites were food limited, because they were not consuming enough carbon to meet their DO demand, whereas carbon consumed on inshore reefs was in excess of DO demand. It is likely that these offshore sponges take advantage of feeding selectivity and transient availability of higher levels of LPOC, detritus, or DOC to meet their metabolic needs and to grow. Optimal foraging on potential food sources was previously demonstrated for *X. muta*, with sponges maximizing their nutritional gains by shifting their diet based on the relative availability of preferred vs. less-preferred food types (McMurray et al. 2016). In the Red Sea, relative and absolute concentrations of the different components of TOC may be subject to temporal variability ranging from daily to seasonal scales (e.g., Raitsos et al. 2013; Silva et al. 2018), as has been described for the Mediterranean (Ribes et al. 1999). Indeed, this variability is evident in the individual variation in specific filtration rates of TOC and DO among the sponges sampled on offshore reef sites in this study. Depending on the extent of food limitation, sponges may shrink in size, something that was documented for *X. muta* on time-series plots in the Florida Keys (McMurray et al. 2008).

The present study corroborates the implicit hypothesis of Wilkinson and Cheshire (1990) that an inshore–offshore gradient of food availability affects the abundance and nutritional status of sponges on coral reefs by demonstrating that this gradient exists and that it influences sponge nutrition. The comparisons of Wilkinson and Cheshire (1990) extended beyond local gradients of food availability to inter-ocean differences, specifically between sponge communities of the GBR (Pacific) and Belizean (Caribbean) barrier reef systems (Wilkinson 1987; Wilkinson and Cheshire 1990). We know that *X. testudinaria* and *X. muta* are closely related barrel sponge species (Swierts et al. 2017); therefore, how does food availability and feeding compare for these two species in the Red Sea and Caribbean? Mean DOC concentrations at inshore and offshore reef sites in the Red Sea were generally higher and lower, respectively, compared to mean ambient concentrations reported for sites in the Caribbean (Table 2); however, the relative quality of DOC (proportion of metabolically usable, labile, and refractory metabolites) among these locations remains unknown. Further, there are no clear patterns of POC availability between the two regions, or of sponge-mediated POC or DOC flux for the two barrel sponge species. As discussed in Pawlik et al. (2018), this is not particularly surprising, given the high intraspecific variability in sponge DOC uptake reported in the literature. Additionally, seasonal variation in POC availability may confound comparisons between the two regions. It does, however, appear that DO demand by *X. testudinaria* in the Red Sea is approximately double than that measured for *X. muta* in the Caribbean (Hoer et al. 2018; Table 2). This difference is likely due to the higher pumping rates observed for *X. testudinaria* in this study (Table 1) relative to the value reported for *X. muta* (0.045 L s^{-1} L_{sponge}) by Hoer et al. (2018). Again, we speculate that enhanced pumping may be evidence of optimal foraging for POC when either the quantity or quality of DOC is insufficient to meet nutritional demands (McMurray et al. 2016). This phenomenon may represent an intraspecific adaptation that is more broadly evident across the spectrum of HMA and LMA sponge species, with HMA sponge species exhibiting lower pumping rates to focus on DOC uptake, whereas LMA species pump faster to enhance uptake of POC (McMurray et al. 2018).

While this study provides evidence of food limitation of sponges based on changes to food availability and DO concentrations in seawater using InEx experiments, more definitive assessments of food-limitation require longer term growth experiments that integrate over the variability in food resources at a given site, as discussed in the preceding paragraph. Indeed, recent deliberations over the relative importance of food limitation and bottom-up and top-down control of sponge communities in the Caribbean have focused on studies of sponge growth over periods ranging from months to years (Pawlik et al. 2018). Nevertheless, the very short-term experiments performed in the present study are the latest in a long tradition of assessing the energetics of sponge feeding (Reiswig 1974, 1981; Hoer et al. 2018), which is essential to understanding food availability, consumption and diet preferences.

Modeled after the microbial loop hypothesis that helped to explain carbon flow in the water column (Azam et al. 1983), the sponge-loop hypothesis (de Goeij et al. 2013) has furthered our understanding of carbon cycling on coral reefs. Much of the research used to support the sponge-loop was done with encrusting species that are found in reef interstices, including species from the Red Sea (Rix et al. 2016). Similar to findings for encrusting species (de Goeij et al. 2008), but in contrast to the emergent species studied to date (Yahel et al. 2003; Hoer et al. 2018), *X. testudinaria* on inshore reefs were found to take up TOC in excess of respiratory demands; however, in contrast to encrusting species, *X. testudinaria* was not found to export carbon back to the benthos in the form of detritus. These findings are consistent with those for emergent sponge species in the Caribbean, including *X. muta* (McMurray et al. 2016, 2018). As an alternative to detritus production, emergent sponges incorporate carbon in the form of growing sponge tissue, which is then available to sponge predators, including turtles, angelfishes and parrotfishes (Pawlik et al. 2018). In the present study, one sponge individual had a negative detrital flux, but the concentration of detritus generated was negligible relative to ambient detrital concentrations.

It should be noted, however, that this study relied on indirect calculations of detritus, making it impossible to distinguish sponge-generated detritus from incurrent detritus that was not consumed by the sponge. As indicated in McMurray et al. (2018), net consumption of detritus by *X. testudinaria* is not consistent with the penultimate component of the sponge-loop hypothesis as originally proposed (de Goeij et al. 2013), and the production of sponge biomass is the more reasonable alternative (McMurray et al. 2018).

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Conflict of Interest

None declared

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